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Nutrient and chemical evaluation of raw seeds of *Xylia xylocarpa*: an underutilized food source

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Some nutritional and antinutritional characteristics of *Xylia xylocarpa* seeds were studied. The mature seeds contained 29·5% crude protein, 14·78% crude fat, 8·02% crude fibre, 5·11% ash and 42·6% crude carbohydrates. The seeds appeared to be a good source of potassium, magnesium, phosphorus and iron. The major components of the total seed proteins were globulins and albumins; the latter constituting 32% of the total extractable proteins. The seed lipids contained a high proportion of unsaturated fatty acids with linoleic acid (51·3%) as the predominant fatty acid. The total seed proteins were rich in most of the essential amino acids and they were deficient only in cystine and methionine. The albumins contained higher levels of isoleucine, cystine, methionine, tryptophan and threonine and lower levels of leucine, lysine, phenylalanine, tyrosine and valine compared to the globulins. Antinutritional substances such as total free phenols, tannins, phytic acid, hydrogen cyanide, trypsin inhibitor and phytohaemagglutinating activities were also analysed besides in-vitro protein digestibility.

INTRODUCTION

Large segments of the population in developing and underdeveloped countries suffer from protein malnutrition due to inadequate protein quantity and/or quality in their diets. Projections based on current trends in human population and in protein supply indicate that the gap will become increasingly wide in years to come (Mayer, 1976). Though there is an urgent need of proteins to meet the nutritional requirements of the ever-increasing population, the available and cheap protein resources have been relatively unexplored (Prakash & Misra, 1988). Nowadays the research efforts are being directed to this area to identify the alternate and cheap sources of proteins against the high cost of animal proteins. Information on the nutritional composition of some of these potential protein sources, including tribal pulses and their possible utilisation as human food, is inadequate. *Xylia xylocarpa*, studied here, is widespread in the forests of southern and north eastern India. The roasted seeds of this tree plant are known to be eaten by the tribal sects, Gonds, Santals and poor families in central India. Since there is no information on its nutritional and chemical qualities, in the present study an attempt has been made to decipher the chemical composition and understand its nutrient potential.

MATERIALS AND METHODS

Source of seed

Seeds of *Xylia xylocarpa* were collected from the Mukkali Reserve Forest (Palghat District, Kerala, India). The seeds were dried in the open sunlight after collection for 2 days. The dried seeds were cleaned thoroughly and any foreign matter and immature seeds were removed.

Proximate composition

The moisture content was determined by drying 50 transversely cut seeds in an oven at 80°C for 2 h and it is expressed on a percentage basis. The seeds were powdered separately in a Wiley Mill to 60 mesh size. The fine seed powder so obtained was stored in screw-cap tubes at –4°C and used for further analysis. The crude protein content was calculated by multiplying the percentage Kjeldahl nitrogen (Humphries, 1956) by a factor of 6·25. The contents of crude lipid, crude fibre and ash were analysed by AOAC (1970) methods. The nitrogen-free extracts (NFE) or total crude carbohydrates were calculated by difference. The energy content of the seeds was estimated in kJ by multiplying the percentages of crude protein, crude fat and NFE by factors of 16·7, 37·7 and 16·7, respectively (Siddhuraju *et al.*, 1992).

Mineral analysis

From the triple acid-digested sample, all the minerals except phosphorus were analysed by atomic absorption spectrophotometer (Perkin–Elmer 5000) (Issac & Johnson, 1975). Phosphorus content in the triple acid-digested extract was determined colorimetrically by the method of APHA (1980).

Extraction and estimation of total seed proteins and seed protein fractionation

The total (true) proteins were extracted by the method of Basha *et al.* (1976) with slight modification (ethanol treatment was omitted in order to save the prolamin fraction). The extracted proteins were purified by precipitation with cold 20% trichloroacetic acid (TCA) and estimated by the method of Lowry *et al.* (1951). The albumin and globulin fractions of seed proteins were extracted and separated according to the method of Murray (1979). From the residual pellet, the prolamin protein fraction was extracted by treating the pellet with 80% ethanol (1:10 w/v) overnight. After centrifugation (20000 $\times g$ for 20 min at room temperature) the supernatant containing prolamins was air-dried and dissolved in 0·1 M in NaOH. The resulting pellet was extracted with 0·04% NaOH (1:10 w/v) overnight and centrifuged at 20000 $\times g$ for 20 min at room temperature. The supernatant thus obtained was designated as glutelins. All the four fractions so obtained were precipitated and washed with cold 10% TCA. All samples were redissolved in 0·2 M NaOH and protein content was determined by the Lowry *et al.* (1951) method.

Amino acid analysis

The total seed proteins and protein fractions, albumins and globulins were extracted by following the methods of Basha *et al.* (1976) and Murray (1979), respectively, and purified by cold 10% TCA precipitation. Known amounts of purified total seed proteins and protein fractions, albumins and globulins, were acid-hydrolysed with 6 N HCl at 110°C for 24 h in Vacuum-sealed tubes. The amino acid analysis was performed using an automated precolumn derivatisation with *o*-phthaldialdehyde (OPA) using revere-phase HPLC, Model-23250. The cystine content of protein samples was obtained separately by the method of Liddle and Saville (1959). For the determination of tryptophan content of protein, aliquots containing known amounts of protein were dispersed into glass ampoules together with 0·75 ml of 5 M NaOH. The ampoules were flame-sealed and incubated at 110°C for 18 h. The tryptophan contents of the alkaline hydrolysates were then determined colorimetrically by the method of Spies & Chambers (1949). The contents of different amino acids recovered are presented as g/100 g proteins and are compared with the FAO/WHO (1990) reference pattern. The essential amino acid score was calculated as follows.

$$\text{Essential amino acid} = \frac{\text{g of essential amino acid in 100 g of the test protein}}{\text{g of essential amino acid in 100 g FAO/WHO reference pattern}} \times 100$$

Fatty acid analysis

The total lipid was extracted from the seed flour according to the method of Folch *et al.* (1957) using chloroform and methanol mixture in the ratio of 2:1 (v/v). Methyl esters were prepared from the total lipids by the method of Metcalfe *et al.* (1966). Fatty acid analysis was performed by gas chromatography (Shimadzu, Model-R 1A) using an instrument equipped with a flame-ionisation detector and a glass column (2 m × 3 mm) packed with 1% diethylene glycol succinate on chromosorb W (silanised 80/00 mesh). The carrier gas was nitrogen, at a flow rate of 32 ml/min. The column temperature was 190°C. Peaks were identified by comparison with authentic standards, quantified by peak area integration and expressed as weight percentage of total methyl esters; the relative weight percentage of each fatty acid was determined from integrated peak areas.

Determination of *in vitro* protein digestibility

The *in vitro* protein digestibility of raw seed sample was estimated using the multienzyme method of Hsu *et al.* (1977)

Analysis of antinutritional factors

The antinutritional factors, total free phenols (Sadasivam & Manickam, 1992), tannins (Burns, 1971) and hydrogen cyanide (HCN) (Jackson, 1967) were quantified. The colorimetric procedure of Wheeler and Ferrel (1971) was followed to estimate phytic acid. Trypsin inhibitor activity was determined by the enzymic assay of Kakade *et al.* (1974). One trypsin unit is expressed as an increase of 0·01 absorbance unit per 10 ml of reaction mixture at 410 nm. Lectin activities of the albumin and globulin fractions were quantified by using the microtitre haemagglutination method described by Tan *et al.* (1983).

RESULTS AND DISCUSSION

The data on proximate composition of *Xylia xylocarpa* seeds are shown in Table 1. They contain higher amounts of crude protein and crude fat than food legumes such as black gram (Khan *et al.*, (1979) pea, cowpea and chickpea (Meiners *et al.*, 1976*a*). Nonetheless, the crude protein alone is comparable with some of the tribal pulses such as *Mucuna utilis* (Ravindran & Ravindran, 1988) and *M. gigantea* (Rajaram & Janardhanan, 1991). Due to the lipid-rich nature, the seeds have a higher energy value than the commonly cultivated pulse crops (Kuzayli *et al.*, 1966).

Table 1. Proximate composition and mineral contents of the seeds of *Xylia xylocarpa*[a]

	Proximate composition (g/100 g seed flour)		Mineral composition (mg/100 g seed flour)
Moisture	5·16 ± 0·16	Na	70·2 ± 0·81
Crude protein (Kjeldahl N × 6·25)	29·53 ± 0·86	K	1016 ± 11·41
		Ca	124 ± 1·18
Crude fat	14·78 ± 0·86	Mg	306 ± 1·70
Crude fibre	8·02 ± 1·13	P	342 ± 2·68
Ash	5·11 ± 0·87	Mn	3·12 ± 0·40
NFE (Nitrogen-free extract or total crude carbohydrates)	42·6	Fe	8·70 ± 0·53
		Cu	2·87 ± 0·39
Energy content (kJ/100 g DM)	1761	Zn	1·32 ± 0·08

[a]Mean ± standard error of three replicates, expressed on a dry weight basis.

Mineral analyses (Table 1) reveal that the contents of sodium, calcium, magnesium, manganese and copper seem to be higher than some of the commonly cultivated legumes (Meiners *et al.*, 1976*b*). *Xylia xylocarpa* seeds, like most legumes appear to be a rich source of potassium.

Seed protein fractionation of *X. xylocarpa* (Table 2) shows that the globulins and albumins together constitute the bulk of (87%) seed proteins as in many other legumes (Boulter & Derbyshire, 1976; Rajaram & Janardhanan, 1991) and the percentage distributions of both the aforesaid protein fractions are comparable with that of *Vigna trilobata* (Siddhuraju *et al.*, 1992). The ratio of albumin to globulin is quite high compared with several other legumes, viz., pigeonpea (Singh & Jambunathan, 1980), chickpea (Dhawan *et al.*, 1991) and ricebean (Rodriguez & Mendoza, 1990).

Table 2. Content of total (true) protein and protein fractions of *Xylia xylocarpa* seeds[a]

Protein fraction	g/100 g seed flour	g/100 g seed protein
Total protein (true protein)	24·32 ± 0·80	100
Albumins	7·86 ± 0·17	32·32
Globulins	13·3 ± 0·60	54·77
Prolamins	0·92 ± 0·07	3·78
Glutelins	2·22 ± 0·10	9·13

[a]Mean ± standard error of three replicates, expressed on a dry weight basis.

The amino acid profiles of the purified total seed proteins, protein fractions, albumins and globulins, and their essential amino acid score are presented in Table 3. The potential food value of the seed proteins as source of amino acids can be justified by comparison with the FAO/WHO essential amino acid requirement pattern (FAO/WHO, 1990). The data on amino acid profiles of the total purified seed proteins reveal that the sulphur-containing amino acids, cystine and methionine, and lysine are the limiting amino acids, whereas the other essential amino acids, leucine, isoleucine, valine, phenylalanine, tyrosine, threonine

Table 3. Amino acid composition of the total seed proteins, protein fractions, albumins and globulins of *Xylia xylocarpa* (g/100 g protein)

Amino acid	Total seed proteins	Essential amino acid score	Albumins	Essential amino acid score	Globulins	Essential amino acid score	FAO/WHO (1990) recommended pattern
Aspartic acid	13·31		10·42		12·70		
Glutamic acid	15·26		12·71		20·28		
Alanine	4·60		5·34		3·86		
Valine	4·23	121	5·18	148	5·80	166	3·50
Glycine	6·17		5·41		4·36		
Arginine	8·14		4·90		7·47		
Serine	3·02		6·14		5·91		
Cystine	0·94	92	1·85	139	1·30	85·2	2·50
Methionine	1·36		1·62		0·83		
Threonine	4·86	143	5·17	152	4·16	122	3·40
Phenylalanine	7·30	166	3·78	103	5·51	142	6·30
Tyrosine	3·14		2·68		3·45		
Isoleucine	3·18	114	4·71	168	4·23	151	2·80
Leucine	8·04	122	6·93	105	7·68	116	6·60
Histidine	3·05		3·77		3·32		
Lysine	5·71	98·4	4·84	83·4	6·18	107	5·80
Tryptophan	1·36	124	1·22	111	0·92	83·6	1·10
Proline	ND[a]		ND		ND		

[a]ND, not determined.

and tryptophan, are found to be relatively high when compared with the WHO requirement pattern (FAO/WHO, 1990). The levels of valine, sulphur-containing amino acids and tryptophan of *Xylia xylocarpa* seem to be nearly equal to that of soya bean (Boulter & Derbyshire, 1976). The albumin proteins reveal higher contents of methionine and cystine than globulins, which is in agreement with the findings in chickpea, pea and mungbean (Bhatty, 1982). When compared with the FAO/WHO recommended pattern, except for lysine, all the other essential amino acids are present in more than adequate levels. The contents of lysine, phenylalanine, tyrosine and valine in globulin proteins, appear to be higher than albumins and the WHO reference pattern; whereas methionine, cystine and tryptophan seem to be the limiting amino acids.

The fatty acid composition of the total lipids of *X. xylocarpa* is given in Table 4. The seeds contain a large proportion of unsaturated fatty acids (67·7%) as in the case of some edible legumes such as *Phaseolus vulgaris* and *Vigna unguiculata* (Omogbai, 1990). The high concentration of linoleic acid (51·3%) is comparable with that of other commonly consumed legumes like chickpea, soya bean and horse gram (Salunkhe *et al.*, 1982) and is nutritionally desirable.

The relatively low levels of *in-vitro* protein digestibility in the legume seeds are due to the presence of globulins as the major storage proteins which are quite resistant to the attack by proteolytic enzymes *in-vitro* (Liener & Thompson, 1980). Nevertheless the *in-vitro* protein digestibility of raw *X. xylocarpa* seed flour seems to be high (80·75%) and is comparable with that of raw seeds of green gram (Reddy & Gowramma, 1987) and higher than that of soya bean (Gross, 1982).

Though food legumes are important sources of dietary protein in the developing countries, their acceptability and utilisation has been limited due to the presence of relatively high concentrations of certain antinutritional factors (Nowacki, 1980). Some of the antinutritional factors such as protease inhibitors, lectins, tannins, goitrogens, cyanogens, antivitamin factors and amylase inhibitors constitute the heat-labile antinutritional factors (Liener, 1980), whereas toxic amino acids, cyanogenic glucosides, saponins, flavones, isoflavones and alkaloids form the heat-stable antinutritional factors (Nowacki, 1980). The data on antinutritional factors are presented in Table 5. The content of total free phenols in *X. xylocarpa* appears to be low when compared with *Phaseolus lunatus* (Egbe & Akinyele, 1990) and *Cajanus cajan* (Singh, 1988). The levels of tannins present in the seeds of *X. xylocarpa* appears to be low compared with the cultivated legumes such as green gram, cowpea, pigeonpea and black gram (Khan *et al.*, 1979; Rao and Deosthale, 1982). Tannins are known to inhibit the activities of digestive enzymes (Jambunathan & Singh, 1981) and hence the presence of even a low level of tannin undesirable from a nutritional point of view. However, in legumes, soaking and cooking processes are known to eliminate phenols and tannins completely (Singh, 1988).

The phytic acid content detected in the present study (360 mg/100 g) is not unexpected in a legume and is of the same order as those reported earlier (Reddy *et al.*, 1982) for grain legumes. Phytate P is known to be the primary storage form of P in mature legume seeds. The high content of phytate is of nutritional significance as, not only is the phytate P unavailable to humans, but it also lowers the availability of many other essential minerals (Reddy *et al.*, 1982). The phytate could, however, be substantially eliminated by processing methods such as soaking and cooking (Reddy *et al.*, 1982).

The present study also reveals the presence of HCN in *X. xylocarpa* seeds. The level observed is much lower than those found in the safe varieties of lima bean (Conn, 1973) and *Mucuna utilis* (Ravindran & Ravindran, 1988) and comparable to that of *Phaseolus vulgaris* (Montgomery, 1980) and is probably too low to cause any concern since cooking is known to reduce it significantly (Sathe & Salunkhe, 1984). The trypsin inhibitor activity is 32·7 TIU/mg protein and it exhibits lower inhibitor activity than various edible legumes

Table 4. Fatty acid composition of *Xylia xylocarpa* seed lipids

Fatty acid	Percentage
Myristic acid (C14:0)	0·62
Palmitic acid (C16:0)	22·14
Stearic acid (C18:0)	7·17
Oleic acid (C18:1)	10·13
Linoleic acid (C18:2)	51·28
Linolenic acid (C18:3)	6·31
Arachidic acid (C20:0)	1·21
Others (Unknown)	1·14

Table 5. Levels of some antinutritional factors present in the raw seeds of *Xylia xylocarpa*

Component	
Total free phenols (%)[a]	0·43 ± 0·04
Tannins (%)[a]	0·21 ± 0·02
Phytic acid (mg/100 g seed flour)[a]	361 ± 8·68
Hydrogen cyanide (mg/100 g seed flour)[a]	1·65 ± 0·06
Trypsin inhibitor activity (TIU[b]/mg protein)[c]	32·7
Phytohaemagglutinating activity[c]	

Name of the protein fraction	Erythrocytes from the human blood group	Haemagglutinating activity (HU[d]/mg protein)
Albumins	A	15
Albumins	B	0
Albumins	O	15
Globulins	A	49
Globulins	B	24
Globulins	O	49

[a]Mean ± standard error of three replicates, expressed on a dry weight basis.
[b]TIU, trypsin inhibitory unit.
[c]Values of two independent experiments.
[d]HU, haemagglutinating unit.

such as *Cicer arietinum, Lens esculenta* and *Vigna unguiculta* (Al-Bakir *et al.*, 1982) and lower than those for soya bean (Kanwar *et al.*, 1991) and pigeonpea (Singh & Jambunathan, 1981). In an earlier study with soya beans (Kanwar *et al.*, 1991) it has been reported that cooking eliminates more than 98% of trypsin inhibitor activity.

The globulin proteins of *X. xylocarpa* agglutinate the erythrocytes from the human ABO system without any specificity as in *Mucuna utilis* (Janardhanan & Lakshmanan, 1985) and *M. pruriens* (Mary Josephine & Janardhanan, 1992). Nevertheless, the albumin proteins weakly agglutinate erythrocytes from the human 'A' and 'O' blood groups specifically. Similarly, the albumins in earlier investigations have been shown to agglutinate erythrocytes specifically from human 'B' and 'O' blood groups in *Entada scandens* (Janardhanan & Nalini, 1991) and 'O' blood group only in *Mucuna pruriens* Lucknow germplasm) (Mary Josephine & Janardhanan, 1992). The action of haemagglutinins is to combine with cells that line the intestinal mucosa and cause a non-specific interference with the absorption of nutrients (Liener, 1980).

The present study reveals that the seeds of *X. xylocarpa* are rich in crude protein, crude fat, some minerals, most of the essential amino acids and exhibit high levels of unsaturated fatty acids and *in vitro* protein digestibility. Besides the antinutritional factors detected in the present study are heat-labile and they can be easily eliminated/reduced by ordinary cooking processes.

While taking into account the overall nutrient and chemical composition, the seeds of *X. xylocarpa* may be adopted as the most cheap and potential alternative protein source to alleviate the protein malnutrition among the economically weaker sections of the population, including tribal people living in developing countries such as India after conducting animal feeding experiments.

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